REMARKS

Claims

By the present amendment, three (3) claims have been amended, zero (0) independent claims have been added, zero (0) dependent claims have been added and zero (0) claims have been cancelled. Claims 1-17 are presently pending.

Claim 1 has been amended as required by the February 22, 2007 Office Action to correct a typographical error. Support for this minor nomenclature correction can be found at least in Claim 1 as filed as well as throughout the application as filed including at least paragraphs 002, 003 and 004.

Claims 14 and 17 have been amended to conform with 37 C.F.R. § 1.75(c) and MPEP § 608.01(n) for multiple dependent claims as required by the February 22, 2007 Office Action and suggested by Examiner Moore during the March 16 interview. Support for the present amendments to Claims 14 and 17 can be found in at least in Claims 14 and 17 as filed.

Applicants respectfully submit that the amendments to Claims 1, 14 and 17 comply with 37 C.F.R. § 1.116(b) as they comply with requirements of form expressly set forth in the February 22 Office Action.

RESPONSE TO FEBRUARY 22, 2007 OFFICE ACTION

In the Office Action dated February 22, 2007, Claims 1 and 14-17 received a final rejection under 35 U.S.C. § 103(a) as being unpatentable over Castelhano et al. (US 6,878,716), optionally in view of Engel et al. (Inter. J. Pharm., 2000). As previously mentioned, Examiner Moore and Jamison Lynch held an examiner interview on March 16, 2007 with a follow-up interview between Examiner Moore and the undersigned on March 19, 2007. During the March 19 interview, Examiner Moore indicated that the present rejections under § 103(a) would be withdrawn if the assertion of unexpected results was supported with references showing that the expected increase in the solubility of a mesylate salt over its corresponding base form is not as dramatic as that found in the mesylate salt of currently pending Claim 1.

I. REBUTTING *PRIMA FACIE* OBVIOUSNESS WITH "UNEXPECTED RESULTS"

The Federal Circuit has repeatedly held, and the USPTO has consistently recognized, that *prima facie* obviousness is rebutted by a showing of "unexpected results." As stated by the Federal Circuit, "[o]ne way for a patent applicant to rebut a *prima facie* case of

obviousness is to make a showing of 'unexpected results,' *i.e.*, to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. The basic principle behind this rule is straightforward – that which would have been surprising to a person of ordinary skill in a particular art would not have been obvious. The principle applies most often to the less predictable fields, such as chemistry, where minor changes in a product or process may yield substantially different results." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995).

By supporting the assertion of unexpected results of the compound described in Claim 1, Applicants do not concede or admit to the correctness of the presently pending rejection under 35 U.S.C. § 103(a). Solely for the purposes of expediting prosecution, Applicants assume (without admitting) that the USPTO has established *prima facie* obviousness for Claims 1 and 14-17 so that a showing of unexpected results will provide a basis for withdrawing the current rejections.

The 4-(4-trans-hydroxy-cyclohexyl)amino-2-phenyl-7H-pyrrolo[2,3d] pyrimidine hydrogen mesylate of Claim 1 exhibits unexpected results. As shown in paragraph 30 of the specification, the base form of 4-(4-trans-hydroxy-cyclohexyl)amino-2-phenyl-7H-pyrrolo[2,3d] pyrimidine has a solubility of 0.0059 mg/L in purified water while the α and β polymorphs have solubilities of 77 mg/L and 18.5 mg/L, respectively. Thus, the mesylate form of the α and β polymorphs had an increase in solubility of approximately 13,000 and 3,100 times, respectively, relative to the base form. This enormous increase in solubility of the mesylate forms over the base form is entirely unexpected. Although water solubility is generally increased through salt formation, a person of ordinary skill in the art would not reasonably expect that the water solubility would increase by such a substantial amount by the formation of a mesylate salt.

The unexpected increase in water solubility of the 4-(4-trans-hydroxy-cyclohexyl)amino-2-phenyl-7H-pyrrolo[2,3d] pyrimidine hydrogen mesylate of Claim 1 is fully supported by the four references discussed below. The four references have been attached as an annex to this Response.

In the first reference, Li et al., *Investigations of Solubility and Dissolution of a Free Base and Two Different Salt Forms as a Function of pH*, Pharm. Research, Vol. 22, No. 4, April 2005 (submitted for publication May 12, 2004 which is contemporaneous with the filing date of the present application of April 21, 2004), the solubility of the base form and mesylate salt of haloperidol at various pH levels is provided. The data in the summary table below is taken from Table I (p. 634) of Li et al. and a solubility ratio has been calculated at

each pH. The solubility ratio is the solubility of the mesylate salt divided by the solubility of the base form. The Li et al. data shows that the solubility of the mesylate salt relative to the base varies (depending upon pH) between 0.82 (mesylate salt less soluble than the base form) and 12.32 – far below the solubility ratios of the compound of Claim 1. As stated above, the solubility ratio of the mesylate compound of Claim 1 is approximately 13,000 and 3,100 for the α and β polymorphs, respectively. The various solubility ratios calculated from the Li et al. data show that the creation of the mesylate salt actually hinders solubility (at pH 1.1) or offers limited improvements in solubility over the base. The solubility ratios of Li et al. are two to three orders of magnitude lower than that provided by the two forms of the mesylate compound of Claim 1. In view of the modest solubility increases (and decrease at pH 1.1) seen in Li et al. upon formation of the mesylate salt, the extreme solubility increase of the two forms of the mesylate compound of Claim 1 over the base is entirely surprising and unexpected.

SUMMARY DATA FROM TABLE I OF LI et al.			
pH	Solubility (mg/ml) (mesylate salt)	Solubility (mg/ml) (base form)	Solubility Ratio (mesylate salt/base form)
1.1	0.65	0.79	0.82
2.0	25.06	3.41	7.35
3.1	28.45	4.16	6.84
5.0	30.44	2.47	12.32

The second reference supporting the conclusion that the dramatic solubility increase of the mesylate compound of Claim 1 is unexpected is Bastin et al., *Salt Selection and Optimisation Procedures for Pharmaceutical New Chemical Entities*, Organic Proc. Research and Dev. 2000, 4, 427-35. The data shown below is taken from Table 4 of Bastin et al. Based upon the summary of the data from Bastin et al., it is evident that the increase in solubility going from the base form to the mesylate salt is between 0.3 (mesylate salt being less soluble than the base form) and only 33.0. The various solubility ratios calculated from the Bastin et al. data show that the creation of the mesylate salt decreases solubility at pH 4. For those pH values where the solubility ratio is 1.0 or greater, the solubility increase of the mesylate salt of Bastin et al. relative to its base form is still two to three orders of magnitude less than the solubility increase of the different forms of the mesylate compound of Claim 1.

SUMMARY DATA FROM TABLE 4 OF BASTIN et al.						
pH	Solubility (mg/ml) (mesylate salt)		Solubility (mg/ml) (base form)		Solubility Ratio (mesylate salt/base form)	
	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
1	131.4	204.1	11.6	14.7	11.3	13.9
2	6.11	8.91	0.71	0.89	8.6	10.0
4	0.01	0.02	0.03	0.05	0.3	0.4
6	0.03	0.34	0.01	0.02	3.0	17.0
6.8	0.01	0.02	0.01	0.02	1.0	1.0
Demineralized water	0.33	0.50	0.01	0.02	33.0	25.0

The third reference demonstrating that the solubility increase of the different forms of the mesylate salt of Claim 1 are unexpected is Floyd et al. (US 5,942,510). Floyd et al. discloses (at col. 2, line 7) that the aqueous solubility of the lamotrigine base is only 0.17 mg/ml at 25 °C. However, when the mesylate salt of lamotrigine is prepared, the aqueous solubility increases to 63 mg/ml (col. 3, line 1). The solubility ratio of the mesylate salt to the base in Floyd et al. is about 371 which is substantially lower than the 13,000 and 3,100 fold increase in solubility seen with the with the α and β polymorphs, respectively, of the 4-(4-trans-hydroxy-cyclohexyl)amino-2-phenyl-7H-pyrrolo[2,3d] pyrimidine hydrogen mesylate of Claim 1.

The fourth reference, Basford et al. (US 6,683,085), like the previous three references, again shows that the increase in solubility achieved by a mesylate salt over the base form is many orders of magnitude lower than the increase in solubility of the mesylate salt of Claim 1 over its base. Basford et al. discloses in Table 2 (col. 2, line 63) the water solubility at 22 °C of the base form and mesylate salt form of 4-amino-6,7-dimethoxy-2-(5-methanesulfonamido-1,2,3,4-tetrahydroisoquinol-2-yl)-5-(2-pyridyl)quinazoline. The water solubility of the base form in Basford et al. is 420 μg/ml while the water solubility of the mesylate salt form is 880 μg/ml. The solubility ratio of the mesylate salt form to the base

form in Basford et al. is about 2.1. The Basford et al. solubility ratio is approximately three to four orders of magnitude lower than the increase in solubility seen with the mesylate salt form of Claim 1 relative to its base.

Based upon the foregoing comparisons of the relative solubilities of the mesylate salt to the base form of several compounds, it is apparent that a person of ordinary skill in the art would recognize that a 3,100 and 13,000 fold increase in solubility of the 4-(4-trans-hydroxy-cyclohexyl)amino-2-phenyl-7H-pyrrolo[2,3d] pyrimidine hydrogen mesylate of Claim 1 over its respective base would be a truly unexpected result. Based upon the four references discussed above a reasonable expectation by a person of ordinary skill in the art would be for very little increase in solubility (and perhaps even a decrease) to perhaps as much as a couple hundred fold increase. Thus, the solubility increase of the mesylate salt of Claim 1 over its base form of 13,000 and 3,100 for the α and β forms, respectively, presents truly unexpected results.

II. Attached Documents

For the convenience of the examiner and to expedite prosecution of this application, the following documents, which have been previously cited and discussed herein, have been attached to this Response.

1. Li et al., *Investigations of Solubility and Dissolution of a Free Base and Two Different Salt Forms as a Function of pH*, Pharm. Research, Vol. 22, No. 4, April 2005;
2. Bastin et al., *Salt Selection and Optimisation Procedures for Pharmaceutical New Chemical Entities*, Organic Proc. Research and Dev. 2000, 4, 427-35;
3. United States Pat. No. 5,942,510; and
4. United States Pat. No. 6,683,085.

III. Conclusion

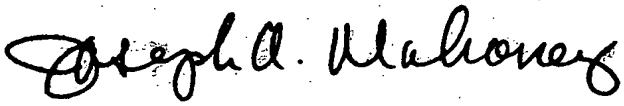
In view of the amendments and arguments set forth herein, Applicant respectfully submits that all existing rejections have been overcome as a clear showing of unexpected results has been made. Withdrawal of the rejection of Claims 1 and 14-17 as obvious over Castelhano optionally in view of Engel is therefore respectfully requested. Upon withdrawal of the currently pending rejections, Applicants respectfully request allowance of all pending claims.

IV. Concluding Remarks

It is to be understood that no admission is made nor implied by the present amendment as to the fact that the cited references may be relevant. Indeed, this amendment is made **solely** to expedite the prosecution of the present application.

Applicants submit that in light of the amendments and arguments provided herein, all pending claims of the present Application are in condition for allowance. Applicants respectfully request entry of the proposed amendment and allowance of the claims. If, in the opinion of the Examiner, a telephone conference would help expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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Salt Selection and Optimisation Procedures for Pharmaceutical New Chemical Entities

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Abstract:

Selection of an appropriate salt form for a new chemical entity provides the pharmaceutical chemist and formulation scientist with the opportunity to modify the characteristics of the potential drug substance and to permit the development of dosage forms with good bioavailability, stability, manufacturability, and patient compliance. Salts are most commonly employed for modifying aqueous solubility, however the salt form selected will influence a range of other properties such as melting point, hygroscopicity, chemical stability, dissolution rate, solution pH, crystal form, and mechanical properties. Where possible, a range of salts should be prepared for each new substance and their properties compared during a suitable preformulation program. Since it is normally possible to fully develop only one salt form, its properties should be appropriate to the primary route of administration and dosage form. An understanding of the influence of drug and salt properties on the finished product is essential to ensure selection of the best salt. The drug properties required for one dosage form may be quite different from those required for another. A well designed salt selection and optimisation study provides a sound base on which to build a rapid and economic product development programme.

Introduction

Modern drug discovery processes involve the screening of vast numbers of compounds that may have been made by the Company's research laboratories over many years. Added to these may be the many thousands of compounds that have been manufactured as libraries of structurally related series by "combinatorial chemistry" techniques. All of these compounds are generally dissolved in dimethylsulphoxide (DMSO) solution and screened in an enzyme- or receptor-based assay system. If the number of "hits" produced is large, the numbers are usually refined by further screening and selection until a manageable number of "leads" is available. Many of these leads will show only weak or moderate activity and further refinement and optimisation is invariably necessary. These optimisation procedures usually involve numerous structural modifications, aided by computational techniques, until a small number (usually 1−5) of highly active "candidates" remain.

These candidates are usually free bases, free acids, or neutral molecules, rather than their salts. Also, because of the generally higher molecular weights of modern drug substances and the increased use of DMSO solutions in the screening processes, it is becoming apparent that there is a tendency towards ever more lipophilic candidates being presented. Frequently, when first proposed as potential development candidates, they are often amorphous or partially crystalline as little effort has been made to investigate formal crystallisation procedures. The need for water-soluble candidates has been recognised[1−4] for many years before the advent of 'combinatorial chemistry.

Investigations into the Possibilities of Salt Formation

When first presented for initial preformulation investigations, normally the amount of drug substance available from Discovery Chemistry rarely exceeds 1 g. To maximize the amount of data gained from such small quantities, semi-micro techniques have been developed and are used regularly within our groups. Invariably, the first information generated for each candidate is the calculated pK_a value of each ionisable group in the molecule.[5−8] This is quickly checked against the value determined experimentally on 1−2 mg of sample by potentiometric titration (e.g., Sirius Model GLpKa apparatus, Sirius Analytical Instruments Ltd.). Knowledge of the pK_a value enables potential salt forming agents (counterions) to be selected, for each candidate, based on lists that are available in the literature.[2,9−11] For the formation of a stable salt, it is widely accepted that there should be a minimum difference of about 3 units between the pK_a value

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Table 1. **Classification of common pharmaceutical salts**

salt class	examples
	Anions
inorganic acids	hydrochloride, hydrobromide, sulfate, nitrate, phosphate
sulfonic acids	mesylate,[b] esylate,[c] isethionate,[d] tosylate,[e] napsylate,[f] besylate[g]
carboxylic acids	acetate, propionate, maleate, benzoate, salicylate, fumarate
anionic amino acids	glutamate, aspartate
hydroxyacids	Citrate, lactate, succinate, tartrate, glycollate
fatty acids	hexanoate, octanoate, decanoate, oleate, stearate
insoluble salts	pamoate (embonate), polystyrene sulfonate (resinate)
	Cations
organic amines	triethylamine, ethanolamine, triethanolamine, meglumine, ethylenediamine, choline
insoluble salts	procaine, benzathine
metallic	sodium, potassium, calcium, magnesium, zinc
cationic amino acids	arginine, lysine, histidine

[a] Based on data from various sources.[9-11] [b] Methane sulfonate. [c] Ethane sulfonate. [d] 2-Hydroxyethane sulfonate. [e] Toluene sulfonate. [f] Naphthalene sulfonate. [g] Benzene sulfonate.

of the group and that of its counterion, especially when the drug substance is a particularly weak acid or base. Occasionally, exceptions may be found where a salt has an acceptable stability, despite there being a smaller difference in the pK_a values.

A microplate technique has been developed for the screening of salts; this involves dissolving approximately 50 mg of sample in a suitable, volatile solvent and adding a fixed volume of this solution, containing about 0.5 mg of sample, into each microplate well. Concentrated solutions of each potential counterion in equimolar proportion, or other appropriate stoichiometric ratio, are prepared and a few microlitres of each is added sequentially to each well. Thus, all of the wells in line 1 (x−direction) will contain the same combination of sample and counterion 1; all of the wells in line 2 contain the same combination of sample and counterion 2, etc. Different, potential crystallising solvents can be investigated methodically in the y−direction. The wells are inspected using an inverted microscope (Leica, Model DMIRB) at regular intervals for the appearance of crystals. Occasionally, crystallisation can be promoted by evaporation of any excess solvent in some wells using a slow stream of dry nitrogen gas.

Once the combinations of counterion and solvent(s) are identified, studies at a slightly larger scale (usually 10−50 mg, occasionally up to 500 mg) can be initiated to confirm the suitability and viability of the crystals. These studies can help identify problems with low melting points, determined by hot-stage microscopy, and hygroscopicity, if processed on a suitable apparatus (e.g., Dynamic Vapour Sorption Analyser, model DVS-1, Surface Measurement Systems Ltd.). Frequently these studies can also give preliminary information on the existence of solvates and hydrates, especially if differential scanning calorimetry (DSC, Mettler Toledo DSC, model 820), thermal gravimetric analysis (TGA, Mettler Toledo TGA, model 850) and hot-stage microscopy are also used in the evaluation process.

In parallel with these studies, a preliminary high performance liquid chromatographic (HPLC) method is quickly developed to give an estimate of the purity of the sample, whilst infrared and other spectroscopic techniques may be used to define the salt and the stoichiometry. Knowledge of the approximate purity is important at this stage as the presence of high levels of some impurities can often hinder crystallisation or alter the polymorphic form obtained.

Therefore, from these preliminary, small-scale studies, a range of potential salt formers and recrysallisation solvents can be quickly identified. Following further scale-up to gram quantities, more comprehensive data can be obtained to evaluate their suitability for use in formulations.

Choice of the Salt Former

Although the choice of salt is governed largely by the acidity or basicity of the ionisable group, safety of the counterion, drug indications, route of administration and the intended dosage form must also be considered. Toxicological and pharmacological implications of the selected salt former must be considered as well as the effects of the parent drug. Salt formers can be subdivided into a number of categories, depending upon their functionality and purpose. Some of the most frequently used examples are listed in Table 1.

The vast majority of salts are developed to enhance the aqueous solubility of drug substances. For weakly basic drug substances, salts of an inorganic acid (e.g., hydrochloride, sulphate, or phosphate), a sulphonic acid (mesylate or isethionate), a carboxylic acid (acetate, maleate or fumarate), a hydroxyacid (citrate or tartrate), or possibly an amino acid (arginine or lysine) could be considered. Hydrochloride salts have often been the first choice for weakly basic drugs, since as a consequence of the low counterion pK_a, salts can nearly always be formed, and recrystallisation from organic solvents is normally straightforward. However, the potential disadvantages of hydrochloride salts may include unacceptably high acidity in formulations (e.g., parenteral products), the risk of corrosion, less than optimal solubility due to the risk of salting out and the potential for poor stability if the drug is acid labile and hygroscopic.[2]

Occasionally, salts may be also prepared to decrease drug substance solubility for use in suspension formulations where very low solubility is necessary to prevent "Ostwald ripening", for taste-masking, or to prepare an extended release product. Embonate salts have been used in suspension

Table 2. [a] **Preformulation studies that are normally considered for comparison of salt forms and parent compound for oral dosage forms**

test	suitable techniques	comments
dissociation constant and basic physico−chemical properties	potentiometry, solubility, UV spectroscopy	determine pK_a for parent drug
melting point	capillary m.pt., hot stage microscopy, differential scanning calorimetry	perform on each salt and compare to parent
aqueous solubility	overnight equilibration at 25 °C; analysis by UV spectroscopy or HPLC	Perform on each salt and compare to parent
pH of solution		Examine pH of saturated solution if quantities permit.
cosolvent solubility	overnight equilibration at 25 °C, analysis by UV spectroscopy or HPLC	Determine solubilities in ethanol, poly(ethylene glycol), propylene glycol and glycerol and compare to parent.
common ion effect on solubility	overnight equilibration at 25 °C in suitable media and analysis by UV spectroscopy or HPLC	compare solubility in demineralized water with 1.2% NaCl for salts and parent
hygroscopicity	use DVS apparatus or expose to various RH values and measure weight gain after 1 week	perform at 53, 93, and 97% RH, and other values of interest; assign hygroscopicity classification to each salt[13]
intrinsic dissolution rate	use Wood's apparatus[14]	compare dissolution rates at various pHs (can provide data on wettability)
crystal shape and appearance	SEM or optical microscopy	Compare crystal habits and levels of agglomeration
particle size	SEM and laser diffraction	Examine particle size distributions.
polymorphism/pseudopolymorphism	recrystallizations, HSM, DSC, TGA	preliminary exploration
powder properties	bulk density measurement	determine Carr's compressibility index
stability	various	perform on parent drug and undertake preliminary tests on appropriate salts

formulations to increase the duration of action (e.g., chlorpromazine embonate). On some occasions, the selection of a salt with only modest aqueous solubility may be more suitable for use in tablet products prepared by wet granulation since the use of highly soluble salts can be detrimental to the granulation process. Depending on the dose required, aqueous solubilities in the range 0.1−1.0 mg/mL will normally be sufficient to satisfy the dissolution requirements for standard, solid, oral dosage forms of drugs with good to moderate potency. However, for parenteral solution products, higher solubilities, perhaps 10 mg/mL or greater, depending on the required dose and dose volume, may be required. For parenteral formulations, the pH of solution (normally within an acceptable range of 3−10 for intravenous solution) should be monitored to help ensure that the formulation will be well tolerated.

Salts are also frequently prepared for the reasons other than solubility modification; it is frequently necessary to prepare a specific salt to either achieve adequate physical stability or for taste masking (e.g., dextropropoxyphene napsylate suspension). Manipulation of drug substance solubility by selection of salts may also be employed to modify the pharmacokinetic profile of the drug (e.g., benzathine penicillin and insulin zinc complexes used in parenteral formulations). Salt formation may be also advantageous where the melting point of the active moiety is low, and it is necessary to mill or micronise the active ingredient to achieve adequate homogeneity. A suitably stable salt may have a melting point that is 50−100 °C higher than the free acid or free base. Also, being more ionic, the crystals are likely to be less plastic and more easily deformed by brittle fracture.

Scale-up of the Formation of Salts

The information from the preliminary crystallisation studies is communicated to the Process Chemistry group, who by this time will have started their investigations into possible manufacturing routes for each of the candidates remaining. At this stage in the development process, Process Chemistry usually aim to quickly manufacture 50−200 g of the one or two candidates that may remain to progress them towards initial clinical evaluation. The manufacturing route may be the same as used by the Discovery Chemistry group but usually is significantly different. The aims of both the Process Chemistry and Preformulation groups for the following 12−18 months is to collaborate extensively to ensure that, for the chosen candidate, there will be a viable synthetic route to the chosen form of the drug substance.

A significant portion of this batch is destined for the preparation of 3−4 g of each of the salts that were thought to be viable from the smaller-scale studies. A similar sized portion of the free base/acid is also taken for comparison purposes. The combination of individual studies undertaken on each of these 3−4 g portions varies depending on the type(s) of dosage form ultimately required for marketing. Occasionally, it may be necessary to undertake a pharmacokinetic evaluation of each salt in comparison with the free acid/base. The dosage forms most commonly used for the drug substances encountered during preliminary clinical investigations are tablets/capsules, inhalation dosage forms and injections.

Table 3. **Tests to be considered for the evaluation of candidate salts**

test to be considered	amount required, mg
Structural Analysis	
mass spectroscopy[a]	1
^{1}H NMR[a]	5
^{13}C NMR[a]	25
Ir spectrum	1
UV spectrum	1
fluorescence spectrum[a]	1
elemental analysis	10
Physicochemical Properties	
melting range	2
pK_a[a]	5
C log *P*/log *P*[a]	5
preliminary polymorphism study	200−500
X-ray diffraction	20
aqueous solubility[b]	100
pH − solubility profile	500
cosolvent solubilities[c]	300
propellant solubility[d]	500
Physical Properties	
hygroscopicity	20
microscopy (SEM/optical)	10
particle size (Malvern)	100
size reduction (sonication)	300
Impurities (hplc)	
related substances[a]	10
degradation products[a]	10
chiral purity[a]	10
Stability Studies	
stability to hydrolysis (pH 2, 7, 10)[a]	15
stability to oxidation (peroxide/peracid)[a]	15
stability to photolysis[a]	15

[a] Determined on free acid/base only. [b] Would include solubility in saline, 5% dextrose and some buffers [c] Also solubilities in complexing agents/surfactant systems where appropriate [d] Propellants and propellant/cosolvent systems for inhalation dosage forms.

Tables 2 and 3 show the types of tests normally chosen, the information that they can produce and the amount of sample normally required for these common dosage forms.

What to Develop: Salt or Free Acid/Base?

The results obtained from each of these tests are tabulated for the free acid/base, together with each of the salts, and discussed in detail between the Formulation Scientists, Preformulation Analysts, Physical Chemists, Process Chemists, and occasionally Pharmacokineticists. The Preformulation Scientists assess the relative merits of each form for use in the proposed clinical formulations and whether the properties such as solubility are adequate to give the high concentrations required in the various pre-clinical formulations. Process Chemistry need to assess the likely yield of each salt, as salt formation creates an additional step in the manufacturing process. Usually, the decision-making process results in the proposal of a single salt for further study, although occasionally it is seen that none of the salts have optimum properties, and two different salts can be proposed for in-depth study. Also, it is occasionally found that the overall properties of the free acid/base are much better than any of the salts. This occurs more frequently where the candidate has a low pK_a value and the resulting salts are less stable than required or when the salts are particularly hygroscopic or when they exhibit complex polymorphism/pseudopolymorphism (hydration or solvation).

These relatively simple investigations give much useful information very quickly; it should be noted, however, that the preliminary polymorphism study is far from the in-depth study that is always undertaken later. This preliminary study uses a range of protic and aprotic solvents of widely differing polarity and will normally show the presence of a stable hydrate or solvate.

Once a decision is agreed upon within the group, a document that gives a précis of the discussions and the basis for the proposal is normally drafted for agreement by senior management. Examples of these salt selection studies are given below:

Example No. 1 (RPR 111423)

RPR 111423 is a candidate drug substance that has been evaluated for the treatment of symptoms related to infection by AIDS. It is a crystalline, very weak base with a pK_a at 4.25. A comprehensive screening of possible salts demonstrated only a monohydrochloride (RPR 111423A) and a mesylate (RPR 111423B) could be isolated as crystalline solids.

It was decided that the free base should be taken through the simple evaluation process in comparison with these two salts. It was expected that the drug substance could be required in the form of tablets or capsules, with an injection form needed for some pre-clinical studies and for the determination of absolute bioavailability in man. Because of its high activity in screening studies, there was a possibility that very low dose oral formulations might be needed. This may require micronised drug substance to enable content uniformity requirements to be met; this micronised material would also be expected to enhance dissolution.

The results from the relatively simple studies undertaken are given in Table 4. The two salts clearly demonstrated the predictable problems associated with a relatively low pK_a value; the salts were quite weak and dissociated to liberate the free base in media with pH values below the pK_a. The very low solubility of the free base resulted in immediate precipitation following dissociation. There was clear evidence for multiple polymorphism for each of the salts, and establishing the existence of a stable polymorph, or a suitable pseudopolymorph, may have been necessary before a decision could be made on which of the two salts could be developed further.

The corresponding results for the free base indicated that it appeared to be the better candidate; it showed no evidence of polymorphism, and it was not hygroscopic. The two major areas that required further investigation were whether it had sufficient solubility in gastrointestinal media and whether it could be micronised. Studies performed on samples of drug substance and on simple capsule formulations demonstrated that the dissolution rates of micronised free base were equivalent or superior to those of the salts under the same conditions.

Table 4. **Comparison of some simple properties of RPR111423 and its two salts**

test	result for RPR 111423 (base)		result for RPR 111423A (hydrochloride)		result for RPR 111423B (mesylate)	
appearance	off-white to cream, crystalline powder		pale yellow, highly agglomerated powder		cream to pale yellow, highly agglomerated powder	
particle size by microscopy, μm	10−100 (large rhombic crystals)		2 × 1 (microcrystalline laths)		7 × 1 (microcrystalline laths)	
melting range, °C	241−244		242		210	
preliminary polymorphism study	no other form detected		at least four polymorphs detected; metastable forms revert to original on standing		at least six polymorphs detected; phase changes detected on grinding or micronisation; reverts to original form on heating	
other thermal behavior	nothing detected		loss of HCl detected at 110−120 °C		nothing detected	
aqueous solubility, mg/mL	at 25 °C	at 37 °C	at 25 °C	at 37 °C	at 25 °C	at 37 °C
- at pH 1	11.6	14.7	25.7	28.2	131.4	204.1
- at pH 2	0.71	0.89	2.51	4.58	6.11	8.91
- at pH 4	0.03	0.05	0.05	0.13	0.01	0.02
- at pH 6	0.01	0.02	0.01	0.02	0.03	0.34
- at pH 6,8	0.01	0.02	0.01	0.02	0.01	0.02
- in demineralized water	0.01	0.02	0.36	0.99	0.33	0.50
pH of saturated solution, at 20 °C, in water	6.50		2.43		2.74	
addition of water to concentrate						
- at pH 2	no changes detected		some precipitation of free base		some precipitation of free base	
- at pH 4	no changes detected		extensive precipitation of free base		extensive precipitation of free base	
hygroscopicity (hygrostat for 14 days)	non-hygroscopic <0.2%w/w water uptake at any RH		slightly hygroscopic 2.3% w/w uptake at 53% RH 22% w/w uptake at 97% RH		moderately hygroscopic 3.7% w/w uptake at 53% RH 32% w/w uptake at 97% RH	

Example No. 2 (RPR 127963)

RPR 127963 is a candidate drug substance that has been evaluated for the treatment of cardiovascular diseases; it is a crystalline, very weak base with a pK_a at 4.10. In common with most similar drug substances intended for the treatment of cardiovascular disease, it was considered that a high-dose (up to 250 mg) solid, oral dosage form and a correspondingly high-dose (up to 50 mg/mL) injection would be ultimately required. In line with our standard protocol, a comprehensive evaluation of possible salts was undertaken, and this demonstrated that five crystalline salts (a hydrochloride, a mesylate, a citrate, a tartrate, and a sulphate) could be readily produced. It was decided to quickly profile each of these salts in comparison with the free base. The results of these studies are given in Table 5.

When the anhydrous free base was evaluated, the existence of an additional mono-, di-, and trihydrate was found quite rapidly. It was shown that all four of these forms could be interconverted under conditions that might be expected to be found in granulation processing. The other potential problem with the anhydrate was the low melting point. In considering the results obtained for the various salts, the solubilities of the citrate and the tartrate were much lower than required for an injectable form and lower than ideal for high dosage formulations. An additional problem for the tartrate salt was the high hygroscopicity. Both of these salts were rejected before completion of the full evaluation. The hydrochloride salt was also shown to have several problems such as lower than ideal solubility, probable multiple polymorphism, and the formation of hydrates.

Thus, the mesylate and the sulphate were the two salts that remained; both had high melting points, excellent aqueous solubility, and were non-hygroscopic. The free base still remained a possible candidate, if a stable hydrate could be found. It was therefore decided to undertake some additional evaluations on these three forms; the results from these are presented in Table 6.

These additional results demonstrate a slight advantage in favour of the sulphate salt because of its greater solubility in cosolvents. This would give the formulator a better chance of achieving a higher dose in an injectable formulation. It was considered that the sulphate salt (RPR 127963E) could be studied further in the more detailed evaluations that would follow over the next few months. The mesylate or the free base (if a suitably stable hydrate could be found) would provide a possible back-up, should unforeseen problems arise.

Example No. 3 (RPR 200765)

RPR200765 is a candidate drug substance proposed for the treatment of rheumatoid arthritis. It is another crystalline, weak base with a pK_a of 5.3 which formed salts with a wide selection of counterions. It was expected that doses of 100−125 mg of RPR200765 in capsules would be required for clinical studies.

Early studies suggested that RPR200765 free base was unacceptable for use in solid, oral dosage forms due to a very poor aqueous solubility of approximately 10 μg/mL and poor bioavailability in animal models. However, RPR200765 would form stable salts with hydrochloride, hydrobromide,

Table 5. **Comparison of some simple properties of RPR127963 and its five salts**

test	result for free base (RPR 127963)	result for HCl salt (RPR 127963A)	result for mesylate salt (RPR 127963B)	result for citrate salt (RPR 127963C)	result for tartrate salt (RPR 127963D)	result for sulfate salt (RPR 127963E)
appearance	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder
particle size (microscopy), μm	1−3 μm (agglomerates of microcrystals)	1−3 μm (agglomerates of microcrystals)	tightly packed spherulites of agglomerated microcrystals 18 μm diameter.	microcrystals (2−3 μm) with some aggregates (70 μm)	rounded agglomerates of microcrystals in domains (70 μm)	aggregates of microcrystals (10−15 μm)
melting range, °C	119−123	166−191 (re-grows at about 166, recrystallizes at 191, then melts at about 275	280.9−282.2	130.2−134.3	198.5−201.6	305.7−308.9
preliminary polymorphism study	several hydrates detected	two monohydrates and one anhydrate	no evidence of polymorphs	stable hemihydrate detected	unstable anhydrate	no evidence of polymorphs
aqueous solubility (25 °C), mg/mL						
- in demineralized water	n.d.[a]	3.92	108	0.83	0.89	~50
- in 0.1 M HCl	n.d.	5.2	50.4	n.d.	n.d.	5.9
- in 0.1 M NaOH	0.020	0.019	0.022	n.d.	n.d.	0.018
- in dextrose 5%w/v	n.d.	2.84	90	n.d.	n.d.	~40
pH of saturated solution	n.d.	2.33	1.76	2.49	2.56	1.32
hygroscopicity	n.d.	non-hygroscopic	non-hygroscopic	non-hygroscopic	very hygroscopic	non-hygroscopic

[a] n.d. = Not determined.

Table 6. **Comparison of additional properties of RPR127963 (anhydrate), its mesylate (RPR 127963B) and sulfate (RPR 12963E) salts**

test	result for free base anhydrate (RPR 127963)	result for mesylate salt (RPR 127963)	result for sulfate salt (RPR 127963)
solubility in cosolvents at 25 °C, mg/mL			
ethanol	190	0.6	0.2
propylene glycol	35.4	0.7	1.7
poly(ethylene glycol) 400	188	0.2	0.2
dimethylsulphoxide	> 500	14	110
N-methylpyrrolidone	> 400	4.4	8.5
glycerol	42	n.d.[a]	2.7
peanut oil	0.18	none detected	none detected
intrinsic dissolution rate, $mg \cdot min^{-1} \cdot cm^{-2}$			
- in water	0.01	n.d.	n.d.
- in 0.01 M HCl	0.35	7.3	7.7
powder flow properties	n.d.	Good, but becomes much worse with increasing humidity	Sticks slightly

[a] n.d. = Not determined.

methanesulfonate, and camphorsulfonate counterions. Aqueous solubility, particle size and shape, powder properties, and polymorphism profile were considered to be the key properties to permit a choice of salt to be made. In addition, it was recognised that the use of some counterions with high molecular weights, would require a large excess of drug substance to achieve the required doses.

Studies demonstrated that the solubility of RPR200765 depended on the amount of drug substance used for the study. This occurred because the counterion reduced the pH of solution and enhanced solubility of the drug base. The mesylate salt consistently produced a higher solubility than any of the other salt forms. The higher solubility resulted in an enhanced dissolution rate of the mesylate salt compared to the other salt forms. The solubility and dissolution rate of the hydrobromide salt was particularly poor. Intrinsic dissolution rate studies on compressed disks could not be carried out because a good compact could not be obtained for most of the salts, and the studies were carried out using drug powder (equivalent to 50 mg free base) in capsules.

Hygroscopicity studies demonstrated that the hydrochloride and hydrobromide salts adsorbed large amounts of moisture on exposure to humidity, resulting in the formation of multiple hydrated forms. The methanesulfonate salt however, was a stable monohydrate form which lost moisture at very low humidity (<10% relative humidity (RH)) but

Table 7. **Comparison of the physicochemical properties of RPR200765 salt forms**

test	result for mesylate salt (RPR 200765A)	result for camphorsulfonate salt (RPR 200765C)	result for hydrochloride salt (RPR 200765D)	result for hydrobromide (RPR200765E)
appearance	off-white to cream, free-flowing powder	white to off-white, crystalline, free-flowing powder	white, free-flowing powder	white to off-white, crystalline, free-flowing powder
MW	566.61	684.79	524.98	569.43
melting range, °C	214	265–267	245–248	276–277
maximum aqueous solubility at 25 °C, mg/mL	39	19.95	16.68	3.29
pH of saturated solution in demineralized water at 20 °C, mg/mL	1.93	2.22	2.16	2.63
hygroscopicity (by DVS)	non-hygroscopic with a stable, monohydrate form	non-hygroscopic	hygroscopic with multiple hydrated forms	hygroscopic with multiple hydrated forms
crystal habit and appearance	individual platelike crystals with some agglomeration.	clusters of highly aggregated, platelike crystals	platelike crystals; individual crystals contain stress lines	loosely agglomerated, flaky material
particle size by microscopy	~45–200 μm in agglomerates of 200–350 μm	crystals ~20–50 μm, clusters ~80–200μm -some larger clusters up to 500 μm	~30–100 μm particles	10–40 μm particles
dissolution studies, drug substance in capsule ($T_{80\%}$, min)				
at pH 2	2.0	6.0	7.4	3.9
at pH 4 (in citrate buffer)	>60% release	>60% release	>60% release	14% release

rapidly re-equilibrated to form the monohydrate form when the humidity was raised. These findings suggested that this salt would be amenable to solid dose formulation and there was little risk of changes in the hydration state on processing or storage under normal conditions. The camphorsulfonate was non-hygroscopic. The results of these studies are outlined in Table 7; in this case very little comparative work was undertaken on the free base due to the poor solubility and bioavailability.

Overall, the studies suggested that the mesylate salt was the favoured form on the basis of its low hygroscopicity, clean polymorphic profile in the preliminary screen, high solubility, and rapid dissolution rates. Another favourable factor supporting the selection of the mesylate salt proved to be the good flow properties which allowed very satisfactory capsule and tablet formulations to be developed.

The Next Steps?

Having evaluated several possible alternatives in the three cases above, using a relatively simple range of tests, a form of the drug substance has been chosen that should be possible to develop further. These simple studies have required 3–4 g of the free base and a similar quantity of each of the salts; the data for all forms normally can be generated in one month, or less. The next steps involve confirmation of the choice, by employing a further range of tests, followed by the optimisation of the form of the salt. A series of tests, analogous to those in Table 2, are used in this evaluation; these tests are given in Table 8.

Optimisation of the Drug Substance Form for Development

Having chosen what should be a reasonably stable form of the salt, free acid, or free base, one of the key activities is to start investigations into which other polymorphic or pseudopolymorphic forms exist. In the short development phase where preclinical administration occurs, only a preliminary screening of these different forms is considered necessary, as it is possible that the compound can be found too toxic for further study. Our team undertakes this on about 500 mg of sample; small portions are recrystallised from anhydrous and hydrated solvents of differing polarity. Any crystalline product recovered is examined by a variety of techniques to determine how many different forms are produced and whether any are hydrates or solvates. Preliminary information on the inter-relationships between the different forms can often be found, even at this early stage.

The remainder of the tests are designed for two main purposes:

To define the various preclinical formulations that are required, to devise analytical methods for these, to determine their stability, and establish shelf lives.

To establish a database for the chosen form and to give an indication of the possibilities for clinical formulations.

To accomplish this, it is normal to request a minimum of 25 g of drug substance, although occasionally more may needed if the drug substance is intended for inhalation and there are difficulties with micronisation.

Table 8. **Tests to be considered for "preclinical phase" (column 2) and in preparation for initial clinical investigation (column 3) for compounds intended for use in oral, injection and inhalation products**

test to be considered	amount required, mg or g	amount required, mg or g
	Physicochemical Properties	
melting range	50 mg	50 mg
optical rotation		1 g
polymorphism	500 mg	25–50 g
X- ray diffraction	20 mg	20 mg
intrinsic aqueous solubility	400 mg	
cosolvent solubilities[a]	500 mg	2 g
propellant solubility[b]		2 g
	Physical Properties	
hygroscopicity	800 mg	-
microscopy (SEM/optical)	100 mg	100 mg
particle size (Laser)	200 mg	200 mg
micronisation	5 g	
specific surface area	2 g (R)	4 g (R)
true density	200 mg (R)	200 mg (R)
bulk powder density	2.5 g (R)	10 g (R)
wettability		1 g
	Impurities (HPLC)	
related substances	10 mg	10 mg
degradation products	10 mg	10 mg
chiral purity	10 mg	10 mg
electrophoresis/TLC	10 mg	
	Stability Studies	
hydrolytic profile (identify degradants)	100 mg	
bulk drug powder		2 g
	Excipient Compatibility	
HPLC, XRPD, and DSC	50 mg	250 mg
	Compression Properties	
for dry powder inhaler only		5 g
	Preclinical Formulation Development	
intra-tracheal suspensions	2 g	
oral solutions/suspensions	2 g	
solutions for nebulization	2 g	
IV solutions	2 g	
other routes (ip/sc)	1 g	-
	Clinical Formulation Development	
	predict suitable dosage forms 3 g	Phase I–IIa[c] 250–1200 g
microbiological controls		*d*
total substance requirements	20–25 g	depends on form and dose

[a] Also solubilities in complexing agents/surfactant systems where appropriate. [b] Propellants and propellant/cosolvent systems for inhalation dosage forms. [c] Develop and specify Phase I formulation – commence stability/compatibility studies. [d] Dependent on drug availability; (R) possible to recycle drug substance for certain other tests.

Once the drug substance is shown to be nontoxic, studies leading to the definition of a suitable series of clinical formulations can begin. For this, we normally expect to have a reasonably clear picture of the inter-relationships between the different forms of the drug substance and should have started to define the most stable form. As more batches are manufactured at increasing scale by Process Chemistry, they are examined using some of the key tests to add information to the database. Also, with the increased availability of drug substance, it is possible to initiate maturation studies as an additional technique to assist in the definition of polymorphism. If the structure of the drug substance has been determined by single crystal X-ray, under certain circumstances it may be possible at this stage to initiate the theoretical search for other polymorphic forms. This is achieved using the Polymorph Predictor software (Molecular Simulations Inc.). This software has been used successfully on several small molecules (molecular weight <500) and predicts theoretical crystal structures and their relative energies. The most stable form has the lowest energy; increasing energy signifies lower stabilities.

As larger quantities of drug substance and samples from different batches become available it is imperative that the variation in basic physical properties (e.g., crystal size and shape, specific surface area, powder flow properties, bulk and tapped density etc.) are studied for each batch. By close liaison with Process Chemistry, it is normally possible to modify the recrystallisation conditions such that greater batch-to-batch uniformity of these physical characteristics can be achieved. Also, these characteristics can often be modified such that they are closer to ideal.

The Negative Aspects of Salt Formation

One of the negative aspects of salt formation is that the percentage active content decreases markedly as higher-molecular weight counterions are used. If the free acid or base has only moderate or low activity, it may be necessary for the patient to have a relatively high dose for a clinical effect. If 20−50% of the weight of the drug substance is due to inactive counterion, the addition of suitable excipients for encapsulation or tableting may result in a powder volume that is too great, even after granulation, to fit successfully into even the largest acceptable capsule shell. This forces the formulator towards a tablet. Even with these formulations, a large tablet (or even multiple, smaller tablets) may be necessary; these do not aid patient compliance.

Other problems that are frequently created, or exacerbated, by salt formation are an increased tendency for the existence or formation of hydrates and polymorphs. Hydrates may be produced in formulations by interaction with water bound to excipients, water in capsule shells, etc.

Final Definition of the Form

As the candidate passes through initial clinical evaluation (Phases Ia and Ib), additional characterisation and refinement of the drug substance form continues in parallel with the finalisation of studies on the drug substance manufacturing process. Close liaison and teamwork between the Process Chemist and the Preformulation Scientist in an exploration of the various possible recrystallisation solvents can often result in further refinement of the crystal properties. An excellent scheme for the final characterisation of solid drug substances, prior to the final regulatory submission, has been described recently.[12] The aim of both the Preformulation and Process Chemistry teams is to finalise the definition of all of the characteristics of the drug substance in readiness for the initiation of Phase IIa clinical trials

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Research Paper

Investigation of Solubility and Dissolution of a Free Base and Two Different Salt Forms as a Function of pH

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Purpose. To evaluate the effect of pH on solubility and dissolution rates of a model weak base, haloperidol, and two different salt forms, hydrochloride and mesylate.

Methods. pH-solubility profiles were determined by using haloperidol base, haloperidol hydrochloride, and haloperidol mesylate as starting materials; concentrated or diluted HCl or NaOH solutions were added to aqueous suspensions of solids to adjust pH to desired values. Intrinsic dissolution rates were determined using intrinsic dissolution apparatus under various pH-stat conditions. Further, approximation of diffusion layer pH was estimated from that of 10% w/w slurries of drug substances in dissolution media, which were used to correlate with intrinsic dissolution rates of haloperidol and its salt forms under different pHs.

Results. pH-solubility profiles of haloperidol base and its HCl salt were similar, while when the mesylate salt was used as starting material, it exhibited a higher solubility between pH 2 and 5. The higher solubility of the mesylate salt at pH 2–5 is attributed to its higher solubility product (K_{sp}) than that of the hydrochloride salt. The pH-solubility profiles indicated a pH_{max} (pH of maximum solubility) of ~5, indicating that the free base would exist as the solid phase above this pH and a salt would be formed below this pH. Below pH 1.5, all solubilities were comparable due to a conversion of haloperidol base or the mesylate salt to the HCl salt form when HCl was used as the acidifying agent. These were confirmed by monitoring the solid phase by differential scanning calorimeter. When their dissolution rates are tested, dissolution rates of the mesylate salt were much higher than those of the free base or the HCl salt, except at very low pH (<2). Dissolution rates of free base and HCl salt also differed from each other, where that of HCl salt exhibits higher dissolution rates at higher pHs. A direct correlation of dissolution rate with solubility at diffusion layer pH at the surface of dissolving solid was established for haloperidol, its hydrochloride, and mesylate salts.

Conclusions. Using pH-solubility and pH-dissolution rate interrelationships, it has been established that diffusion layer pH could be used to explain the observed rank order in dissolution rates for different salt forms. A non-hydrochloride salt, such as a mesylate salt, may provide advantages over a hydrochloride salt due to its high solubility and lack of common ion effect unless at very low pH.

KEY WORDS: basic drug; diffusion layer pH; dissolution; haloperidol; hydrochloride; intrinsic dissolution rate; mesylate; pH; salt forms.

INTRODUCTION

A common approach to improve dissolution of a compound is by forming salts. Early work on dissolution rates of pharmaceutical salts and its impact on bioavailability have been nicely reviewed by Berge *et al.* (1). There are many reports in the literature on the selection of optimal salt forms of new drug candidates (2–4). However, the process of a systematic screening of various potential salt forms of a compound for the identification of an optimal one with desirable physicochemical and biopharmaceutical properties is still hindered by the lack of a comprehensive understanding of their solubility and dissolution behavior. The selection of an optimal salt form purely based on physicochemical properties such as crystallinity, thermal properties, hygroscopicity, and stability may be inadequate due to its inappropriate biopharmaceutical attributes, such as solubility and dissolution rate. Although several investigators attempted to address solubility and dissolution issues in reference to salt selection (4–7), questions that have not been adequately addressed are the following: How will solubility and dissolution of nonhydrochloride salts be influenced after oral ingestion by gastric pH? Will a nonhydrochloride salt provide any advantage over a hydrochloride salt? or will it convert into a hydrochloride salt in the presence of HCl in the stomach without providing a significant advantage? In this report, attempts were made to

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address these questions by using different salt forms of a model drug: haloperidol.

MATERIALS AND METHODS

Materials

Haloperidol free base was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), and its hydrochloride and mesylate salts were synthesized at Novartis Pharmaceuticals Corp. (East Hanover, NJ, USA). Purified and deionized water (Millipore, Bedford, MA, USA) were used in this study. Other reagents used include hydrochloric and methylsulfonic acids from Sigma-Aldrich (St. Louis, MO, USA). All solvents and other chemicals were of analytical reagent grade.

Thermal Analysis

Differential scanning calorimetry (DSC) was used to characterize haloperidol free base, its hydrochloride and mesylate salts. The solid phase after pH-solubility experiments were also characterized. All thermal analysis were carried out using TA Instruments (New Castle, DE, USA) Model 2920 differential scanning calorimeter. Samples (~5 mg) were placed in a flat crimped aluminum pans and heated at a rate of 10°C/min under N_2 purge (50 ml/min). Potential changes in drug substance, such as salt forms, hydration state or polymorphs were monitored.

Determination of pH-Solubility Profile

pH-solubility profiles of haloperidol were determined at 37°C by phase-solubility techniques (8). Initially, excess solids (free base, hydrochloride or mesylate salts) were added to a 10-ml vial containing 5 ml of water. Solubility at different pH values were determined by stepwise titration with HCl or NaOH solutions. After each addition of acid or alkali, the suspension was equilibrated for over 24 h, the pH value was recorded, and an aliquot was removed and filtered using a 0.45-μm AcroDisc filter (Fisher Scientific, Fairlawn, NJ, USA). Haloperidol solutions were analyzed by UV spectrophotometer (HP2940) by diluting them to appropriate analytical ranges. The detection wavelength was 250 nm, and a linearity range between 0.25 to 100 μg/ml was used.

Intrinsic Dissolution Experiments

Intrinsic dissolution rate experiments were conducted in a rotating disk apparatus. Disks of haloperidol drug substances were prepared by directly compressing 200 mg of free base and hydrochloride or 500 mg of haloperidol mesylate in a die at a pressure of 1 ton for 30 s using a hydraulic press (Carver Press, Fred Carver, NJ, USA). The exposed surface area for the resulting disk was 0.5 cm^2. A regular USP dissolution apparatus maintained at 37 ± 0.5°C was used for the dissolution study. Each dissolution vessel contained 500 mL of aqueous dissolution medium maintained at various unbuffered conditions (constant pH at 1.0, 2.0, 3.0, 5.0, and 7.0) by titration of dilute HCl or NaOH solutions. The disk holder (die) was half-immersed into the dissolution medium and rotated at 200 rpm. Samples were withdrawn automatically at 15, 30, 45, 60, 75, 90, 105, and 120 min, filtered, and then analyzed by an online HP 8452A UV Diode Array Spectrophotometer (Agilent, Palo Alto, CA, USA) at the detection wavelength of 250 nm.

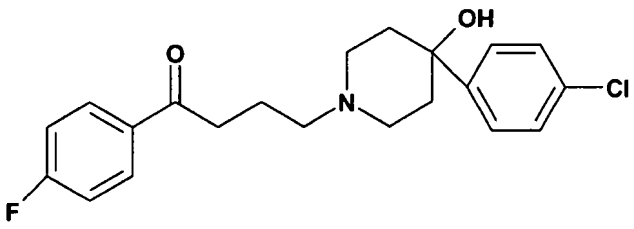

Fig. 1. Chemical structure of haloperidol free base.

Diffusion Layer pH Measurement

Diffusion layer pH of haloperidol free base and its salts were measured by suspending 100 mg of drug substance in 1 ml of each dissolution medium in which the intrinsic dissolution experiments were conducted. The slurry contains 10% w/v solid content and the pH measured were used to approximate the pH at the surface of solid as the diffusion layer thickness approaches zero ($pH_{h=0}$) (8).

RESULTS AND DISCUSSION

pH-Solubility Profiles

Figure 1 shows the structures of haloperidol, a model compound used in this study. Its pK_a value and intrinsic solubility (solubility of nonprotonated form) are 8.0 and 2.5 μg/ml, respectively (Fig. 2). Figure 2 gives the pH solubility profiles of haloperidol when the free base form and the hydrochloride salt were used as starting materials for the determination of solubility, where pH was adjusted by using solutions of HCl or NaOH, as necessary. There is generally a good agreement between the experimental values (symbol) and theoretical prediction (line). Figure 3 gives the pH-solubility profile using haloperidol mesylate as starting material by adjusting pH with HCl or NaOH solutions.

Equations elucidating pH-solubility interrelationships of salt and free base forms of basic compounds were first described by Kramer and Flynn (9). Subsequently, there have been many other reports on both experimental and theoretical aspects of the solubility of basic drugs as a function of pH

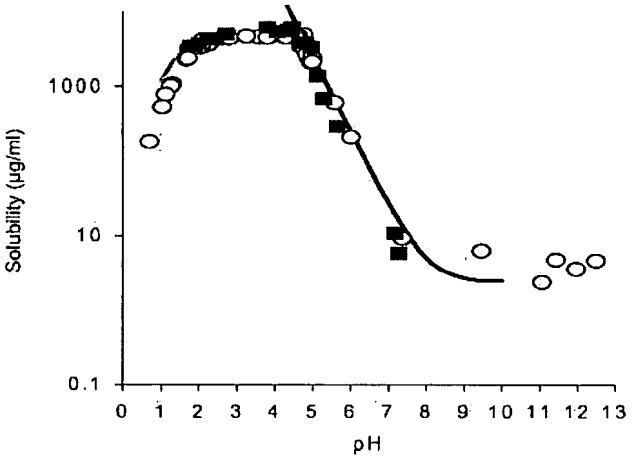

Fig. 2. pH-solubility profile for haloperidol free base (■) and its HCl salt (○).

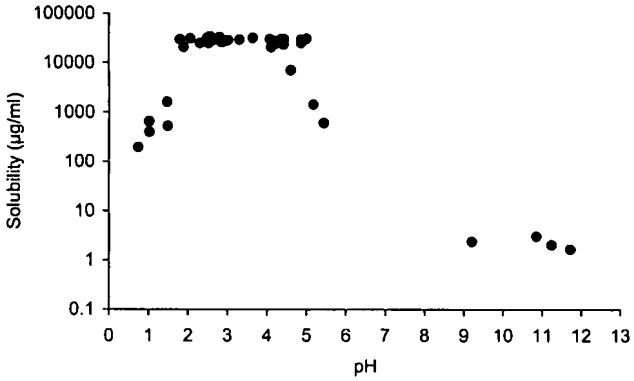

Fig. 3. pH-solubility profile for haloperidol mesylate (●).

(6,10). Essentially, when a basic compound or its salt is dissolved in water, the following equilibrium exists:

$$BH^+ + H_2O \overset{K_a}{\Leftrightarrow} B + H_3O^+ \quad (1)$$

Or,

$$K_a = \frac{[B][H_3O^+]}{[BH^+]} \quad (2)$$

where BH^+ and B represent, respectively, protonated and free base forms of the compound. Therefore, when the salt form is the saturation or equilibrium species, that is, when the salt exists as the solid phase during the determination of the pH-solubility profile, the total solubility (S_T) in the aqueous solution is:

$$S_{T,salt} = [BH^+]_S + [B] = [BH^+]_S \left(1 + \frac{K_a}{[H_3O^+]}\right) \quad (3)$$

where S represents saturation species. On the other hand, when the free base is the saturation species,

$$S_{T,base} = [BH^+] + [B]_S = [B]_S \left(1 + \frac{[H_3O^+]}{K_a}\right) \quad (4)$$

Equations 3 and 4 represent two independent pH-solubility curves and the point where the two curves intersect is generally defined as pH_{max}, the pH of maximum solubility. Therefore, the $S_{T,salt}$ in Equation 3 is for the solubility below pH_{max} and may also be denoted by $S_{T,pH<pHmax}$ to differentiate it from $S_{T,base}$ in Eq. (4). The S_T in Eq. (4) is for the solubility above pH_{max} and may thus be denoted by $S_{T,pH>pHmax}$. Depending on pK_a, intrinsic solubility of the base and solubility of the salt, pH_{max} values may vary. For example, pH_{max} will increase by one unit when any of the following factors changes: an increase in pK_a by one unit, a 10-fold increase in intrinsic solubility, or a 10-fold decrease in the solubility of salt. Therefore, all these factors have profound impact on salt formation.

Figure 2 shows that pH-solubility profiles are similar whether hydrochloride salt or free base was used as starting material. The pH_{max} of haloperidol, where the free base and salt curve intercepts, is around 5, this indicates that when the salt is used as the starting material, it converts to free base if the pH of a suspension is raised above 5. In the same way, the free base converts to the hydrochloride salt when the pH of its suspension is lowered below 5. Therefore, similar species, both in solid phase and in solution, remained in equilibrium at any particular pH irrespective of the use of hydrochloride salt or free base as the starting material, and this explains the similarity in the two profiles. On the other hand, when haloperidol mesylate was used as the starting material, the solubility measured was much higher than that of the hydrochloride salt (Fig. 3). For example, the solubility of the mesylate salt in the pH region of 3 to 5 was in the range of 25 to 29 mg/ml, while that of the hydrochloride salt was in the same pH region is 4.2 to 4.3 mg/ml. This is due to the high solubility of mesylate salt and therefore the higher buffer capacity provided by mesylate. The profile at pH > 5 in Fig. 3 is, however, similar to those in Fig. 2, as the solid phase converted to the free base at pH higher than the pH_{max}. The conversion between different forms is monitored by DSC, which will be discussed at later section.

It may also be noted that in both Figs. 2 and 3, solubility decreased gradually at low pH, when HCl was used to titrate the pH to less than 1.5. This is due to the common ion effect on solubility of the HCl salt. The difference in solubility between hydrochloride and mesylate salts and the common ion effect may be explained based on solubility products of the salts (11). When the pH of an aqueous suspension (slurry) of a free base is lowered by the addition of an acid, the following relationship exists:

$$[B]_S + [BH^+]\uparrow + [X]\uparrow \Leftrightarrow (B)\ \text{solid} \quad (5)$$

where X^- represents the anion species from the acid added. As indicated by the arrows, concentrations of the protonated compound and the counterion increase with the addition of acid. This continues until the concentration of $[BH^+]$ exceeds the salt solubility, at which point the salt, (BH^+X^-) solid, nucleates. Eventually, all the solid base converts to solid salt and the equilibrium shifts to:

$$(BH^+X^-)\ \text{solid} \Leftrightarrow [BH^+]_S + [X^-] \quad (6)$$

The apparent solubility product (K'_{sp}) can be derived from Eq. (6) as follows:

$$K'_{sp} = [BH^+]_S [X^-] \quad (7)$$

In the absence of excess counterion (X^-), $[BH^+]_S = [X^-]$, and therefore, solubility $= \sqrt{K'_{sp}}$.

Otherwise, $[BH^+]_S = K_{sp}/[X^-]$. From the relatively flat solubility profiles of hydrochloride and mesylate salts between pH 3 and 5 (Figs. 2 and 3), the apparent K_{sp} values of the two salts were calculated to be 1.6×10^{-4} M and 2.0×10^{-3} M, respectively. Such a difference in solubility products explains the difference in solubility of various salt forms of a particular compound.

Because HCl solution was used to titrate the pH, counter ion X^- in this case was Cl^-. The decrease in solubility of haloperidol at pH below 3 (Fig. 2) is due to a gradual increase in chloride ion concentration as the pH was lowered. For the mesylate salt (Fig. 3), no decrease in solubility was observed until the pH was lowered to 1.5. This was because the pH was adjusted by using HCl and, therefore, no excess common ion initially existed for mesylate. Conversion from mesylate to

hydrochloride salt form at pH below 1.5 was confirmed by differential scanning calorimetric testing of the solid phase (Fig. 5). The pH at which solubility of the mesylate salt started to decline is related to both the amount of mesylate added at the start of the solubility testing and the amount of hydrochloride acid added to lower the pH (Eq. 8). When there was enough $[H^+][Cl^-]$ in the solution to convert all the mesylate drug substance, the conversion as indicated in Eq. (8) would occur, hydrochloride salt would be obtained at such pH.

$$(BH^+ CH_3SO_3^-)_{solid} \Leftrightarrow [BH^+] + [CH_3SO_3^-] + [H^+] + [Cl^-] \Leftrightarrow (BH^+Cl^-)_{solid}\downarrow + CH_3SO_3H \quad (8)$$

As a consequence of this phase conversion and the consequent susceptibility of the converted solid phase to the common ion effect at lower pH, the solubility of the mesylate salt decrease with a further decrease with pH (Fig. 3; pH < 1.5). Despite the potential for phase conversion, the lack of influence of added HCl on the mesylate salt solubility over a wider gastric pH range (pH 2–5) provides an useful insight into potential advantages of using mesylate and possibly certain other salt forms over the hydrochloride salt.

Thermal Analysis

The differential scanning calorimetry curves of haloperidol free base, its hydrochloride and mesylate salts were shown in Fig. 4. Haloperidol free base has an onset of melting point approximately 151.4°C; that of its hydrochloride and mesylate salts are 230.8°C and 165.2°C, respectively.

Solid phase of haloperidol mesylate after pH-solubility study were also characterized, their DSC curves were shown in Fig. 5. Under pH ~1, the solid phase was characterized to had a similar melting point as that of its hydrochloride salt (Fig. 5A), indicating a conversion from mesylate to hydrochloride had occurred due to the addition of hydrochloride acid, at lower pH, the conversion was also facilitated by the lower K_{sp} of the HCl salt compared with that of the mesylate. When solid phase of mesylate under pH ~4 was tested, a typical DSC profile of mesylate was obtained (Fig. 5B), which confirmed that the high solubility observed between pH 2-5 was contributed by mesylate salt. At pH > pH_{max} (pH_{max} = 5), the pH-solubility curved is theoretically controlled by free base form. When the solid phase of mesylate was tested under pH 6.8, a free base DSC curve was evident (Fig. 5C).

The fact that different solid forms were observed under various pHs, when mesylate was used as starting material to prepare its pH-solubility profile is interesting, but not surprising. As discussed in the previous section, at pH above pH_{max}, free base is thermodynamically the most stable form, total solubility is determined by free base solubility and its ionized species (Eq. 4), whereas at pH below pH_{max}, the salt form is responsible for total solubility (Eq. 3), as the salt is thermodynamically the more stable form. Conversion from mesylate to hydrochloride salt is affected by both pH and chloride ion concentration. As indicated by Eq. (8), chloride ion will replace mesylate counterion under given pH when the K_{sp} of hydrochloride salt is lower than that of mesylate.

Intrinsic Dissolution Rates

Figures 6, 7, and 8 illustrate intrinsic dissolution profiles of haloperidol free base, hydrochloride salt and mesylate salt, respectively, as a function of pH. The individual rates in mg/min/cm^2 are tabulated in Table I. As shown in column 2 of Table I and in Fig. 6, the dissolution of haloperidol free base was highest at pH 2.0, followed by pH 3.1, 1.1, and 5.0, and the rate at pH 7 was so low that it was practically not measurable. In contrast, dissolution rates of the hydrochloride salt were highest at pH 3.1 and 5.0, followed by pH 7.0, 2.0, and 1.1. The

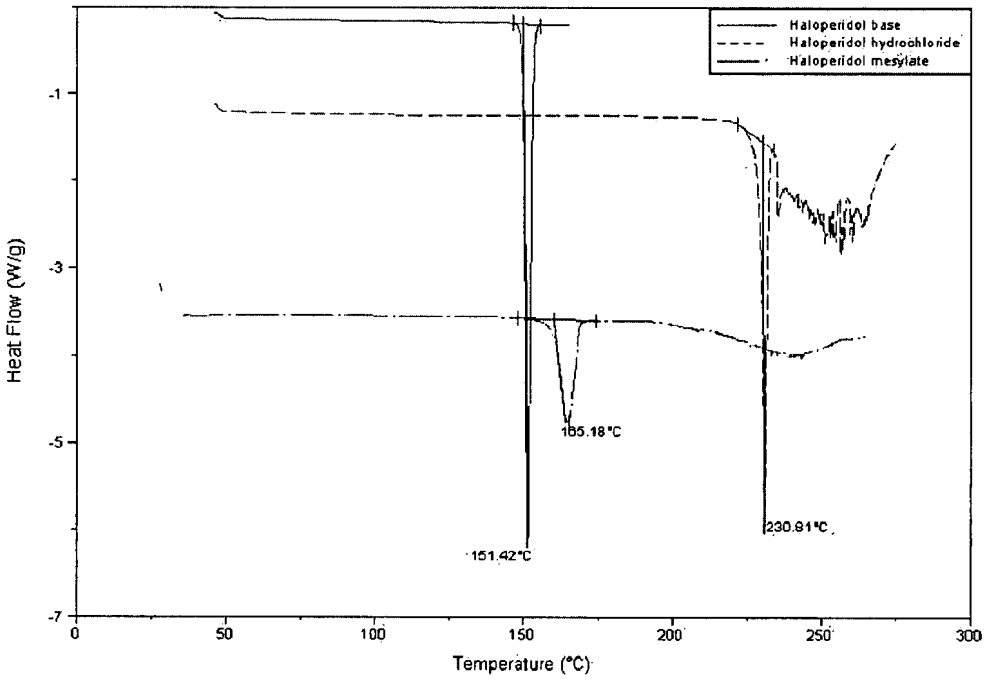

Fig. 4. Comparative DSC curves of haloperidol free base, its hydrochloride and mesylate salts.

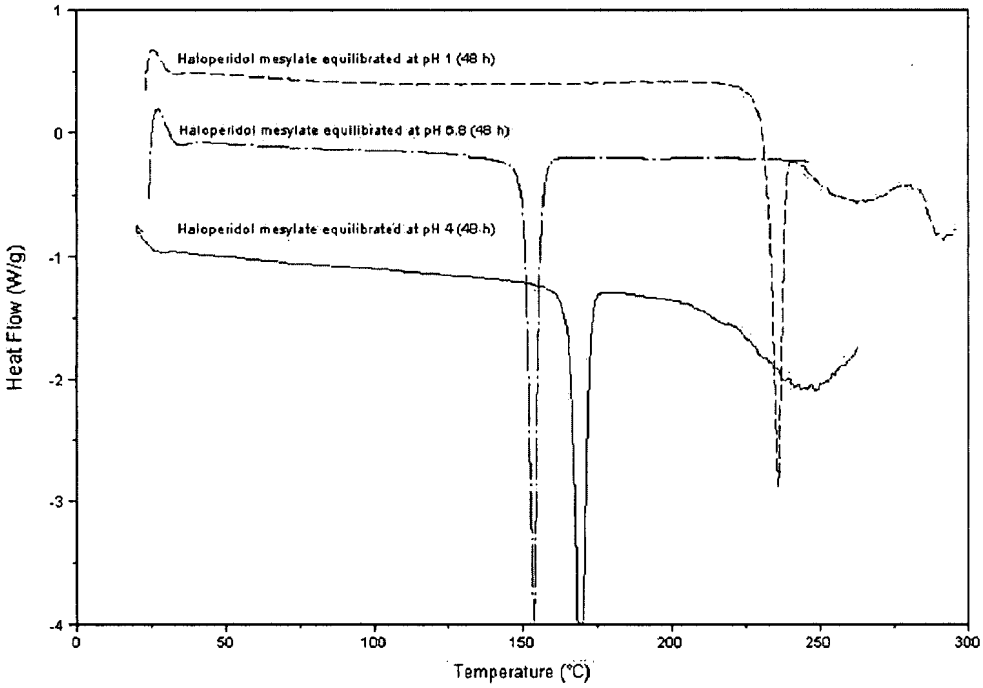

Fig. 5. Comparative DSC curves of solid forms of haloperidol mesylate equilibrated at pH 1 (A), pH 4 (B), and pH 6.8 (C).

dissolution rates of mesylate salt were similar and the highest in the pH range of 3.1 to 7.0, and then decreased at pH 2.0 and 1.1 in a gradual order. Further, the dissolution rates of the mesylate salt in the pH range 2.0 to 7.0 are greater than that of the hydrochloride salt by a factor of approximately 6, which is consistent to difference in their K_{sp}. These seemly random rank order of intrinsic dissolution rates of haloperidol free base, its hydrochloride and mesylate salts can be explained by theory of diffusion layer pH, which will be discussed in the following section.

The dissolution rate (J) of solid per unit surface area is given by:

$$J = \frac{D}{h}(C_S - C_b) \tag{9}$$

where D is the diffusion coefficient of the solute, h is the diffusion layer thickness during dissolution at the surface of solid, C_S is the saturation solubility of the solid in the dissolution medium, and C_b is its concentration in the bulk medium. For haloperidol base and salts, D may be considered to be constant, and, under identical dissolution conditions, h also remains constant. Therefore, under "sink" conditions (C_b < 10% of C_S), Eq. (9) is reduced to:

$$J \sim \frac{D}{h} C_S \tag{10}$$

As a consequence, a ratio J to C_S would also remain constant. However, as it is evident from column 3 in Table I, that is not the case, and the J/C_S ratios varied widely and without any definite order when Cs at the bulk pH is used for calculation.

Previous reports (4,12) demonstrated that it is not the solubility under a bulk pH condition, rather it is the solubility under pH condition at the solid surface in the diffusion layer ($C_{S,h=0}$), that controls dissolution rates of solids in reactive media. The pH at the dissolving surfaces of haloperidol base and salts ($pH_{h=0}$) were, therefore, responsible for the dissolution rates observed. This could be measured by a method described earlier (4,8) and the results are plotted in Fig. 9, where the pH as the diffusion layer thickness approaches zero ($pH_{h=0}$) of haloperidol base and its salts were approximated by concentrated slurry prepared from unbuffered dissolution media. As demonstrated in Fig. 9, diffusion layer pH could differ significantly from that of bulk pH, which can significantly influence drug solubility in the diffusion layer and, as a consequence, the dissolution rate. For instance, when the bulk pH varied between 3 and 7, $pH_{h=0}$ of the mesylate salt remained practically unchanged around pH 3. Because this pH is below the pH_{max}, where the drug solubility is high, the dissolution of the mesylate salt in the pH range of 3 to 7 is also

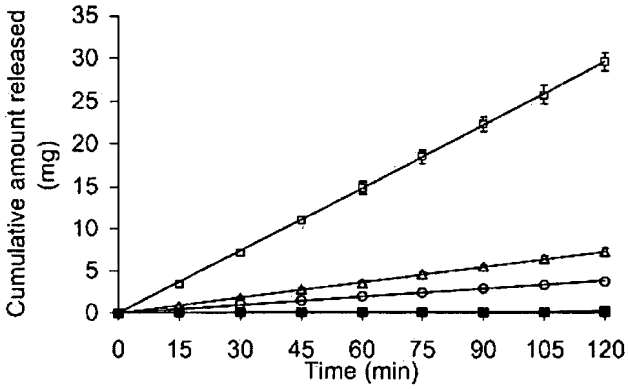

Fig. 6. Intrinsic dissolution rate of haloperidol free base at various pH (-○- pH 1.0, -□- pH 2.0, -△- pH 3.0, -■- pH 5.0). Data presented as mean ± SD; n = 6.

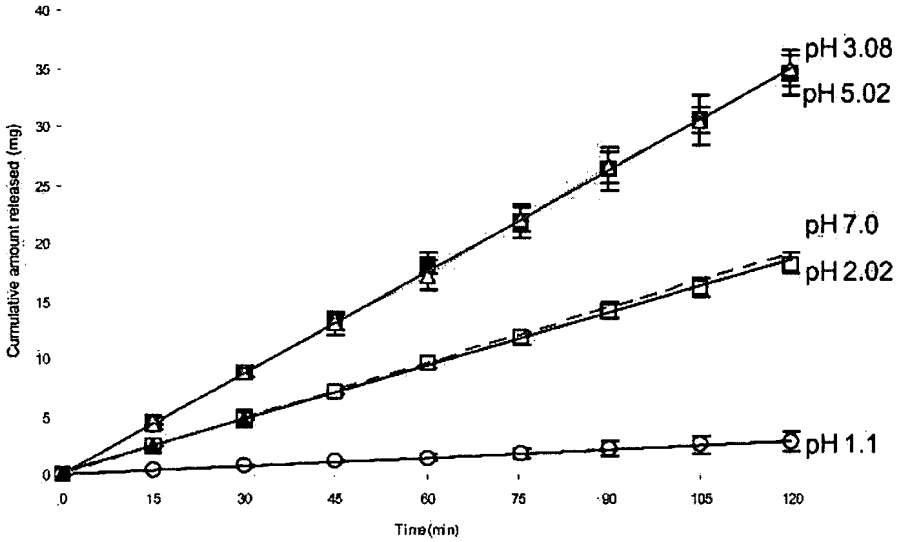

Fig. 7. Intrinsic dissolution rate of haloperidol HCl at various pH (-○- pH 1.0, -□- pH 2.0, -△- pH 3.0, -■- pH 5.0, -▲- pH 7.0). Data presented as mean ± SD; n = 6.

expected to be high. Essentially similar effect was also observed for the hydrochloride salt. On the other hand, the $pH_{h=0}$ in this bulk pH range is above the pH_{max} for the free base, and, therefore, the drug solubility in the diffusion layer and the dissolution rate would be lower.

Given the reason above, the dissolution rate to drug solubility ratios were recalculated using solubilities under pH conditions at solid surfaces. As shown in column 7 in Table I, $J/C_{S,h=0}$ values, calculated as a ratio between intrinsic dissolution rate (Table 1, column 2) and solubility at solid diffusion layer pH (Table I, columns 4 and 5), under different pH conditions and for different solid forms are very similar, slight deviations at other pH conditions could be due a difference in solute activity.

It may be concluded from Figs. 6 to 8 and the data in Table I that although the free base appeared to have a dissolution advantage at a lower pH, e.g., pH 2, the reverse is true at a higher pH, where dissolution rates a salt (hydrochloride or mesylate) could be higher by several orders of magnitude. This is due to the much higher solubility in their diffusion layer pH of the salt forms compare to that of free base. On the other hand, at pH 1.1, all the three forms had similar dissolution rates, this can be explained by the fact that the free base and its hydrochloride and mesylate salt forms have similar diffusion layer pH under this pH. As discussed earlier in pH-solubility section, conversion to HCl salt could help to explain the similarity in their dissolution rates.

In evaluating various free or salt forms of a drug candidate for selecting an optimal form for development, care must be given to studying dissolution rates at multiple conditions. It is also evident that a nonhydrochloride salt with higher drug solubility may have an advantage over the hydrochloride salt. The dissolution rate of haloperidol mesylate remained much higher than that of the hydrochloride salt at a wide

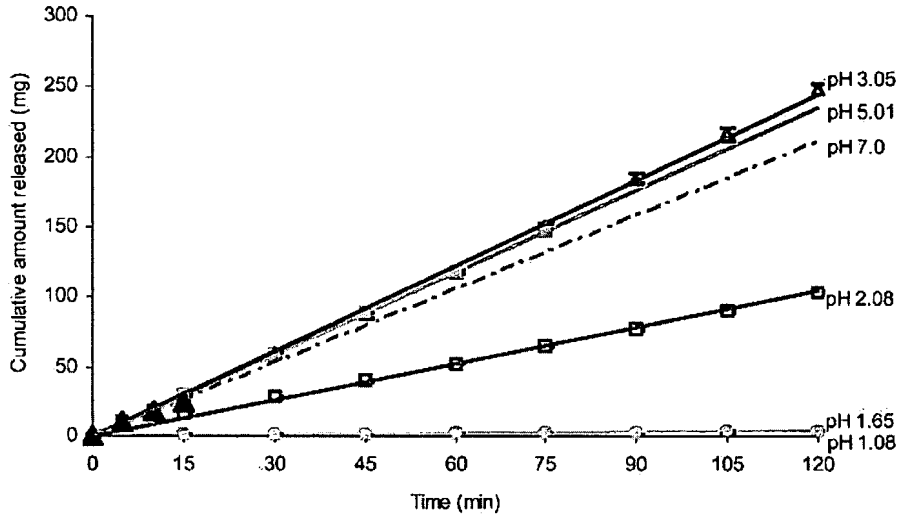

Fig. 8. Intrinsic dissolution rate of haloperidol mesylate at various pH (-○- pH 1.0, -□- pH 2.0, -△- pH 3.0, -■- pH 5.0, -▲- pH 7.0). Data presented as mean ± SD; n = 6.

Table I. Intrinsic Dissolution Rates of Haloperidol Free Base, Haloperidol Hydrochloride, and Haloperidol Mesylate as a Function of pH

pH of dissolution medium	Dissolution rate (J, mg min^{-1} cm^{-2})	Solubility at bulk pH (C_s, mg ml^{-1})	J/C_s (ml min^{-1} cm^{-2})	pH at solid surface ($pH_{h=0}$)	Solubility at solid surface ($C_{s,h=0}$, mg ml^{-1})	$J/C_{s,h=0}$ (ml min^{-1} cm^{-2})
Free base						
1.1	0.032	0.79	0.041	1.11	0.65	0.049
2.0	0.246	3.41	0.072	4.76	3.48	0.071
3.1	0.061	4.16	0.015	5.93	0.73	0.084
5.0	0.002	2.47	0.001	7.00	0.02	0.072
HCl salt						
1.1	0.025	0.79	0.032	0.88	0.56	0.044
1.5	0.062	2.50	0.025	1.37	1.20	0.052
2.0	0.155	3.41	0.045	1.85	2.84	0.055
3.1	0.292	4.16	0.070	3.01	4.16	0.070
5.0	0.291	2.47	0.118	4.89	4.28	0.068
7.0	0.157	0.02	7.140	4.85	2.82	0.056
Mesylate salt						
1.1	0.033	0.65	0.051	1.04	0.65	0.050
1.7	0.115	20.76	0.006	1.5	2.50	0.046
2.0	0.865	25.06	0.035	1.91	20.77	0.042
3.1	2.037	28.45	0.072	2.61	24.91	0.069
5.0	1.962	30.44	0.064	3.02	26.19	0.075
7.0	1.760	0.002	880.000	2.99	28.90	0.061

gastrointestinal pH range. The dissolution rate of the mesylate salt decreased only at pH below 2.

CONCLUSIONS

Depending on solubility products, different salt forms of a basic drug may have different aqueous solubilities. The current study shows that a non-hydrochloride salt, such as a mesylate salt, having aqueous solubility higher than that of a hydrochloride salt may have certain biopharmaceutical advantages. A non-hydrochloride salt is not as susceptible to the common ion effect due to the presence of HCl under gastric pH as a hydrochloride salt, unless it is converted to a hydrochloride salt form at a very low pH and abundance of chloride ion.

Although a free base may also exhibit superior dissolution rates at a low pH, the advantage disappears as the pH increases, and its dissolution rate under intestinal pH condition could indeed be much lower than that of its salt forms. If the total dose of a weak base is not dissolved in gastric pH, a

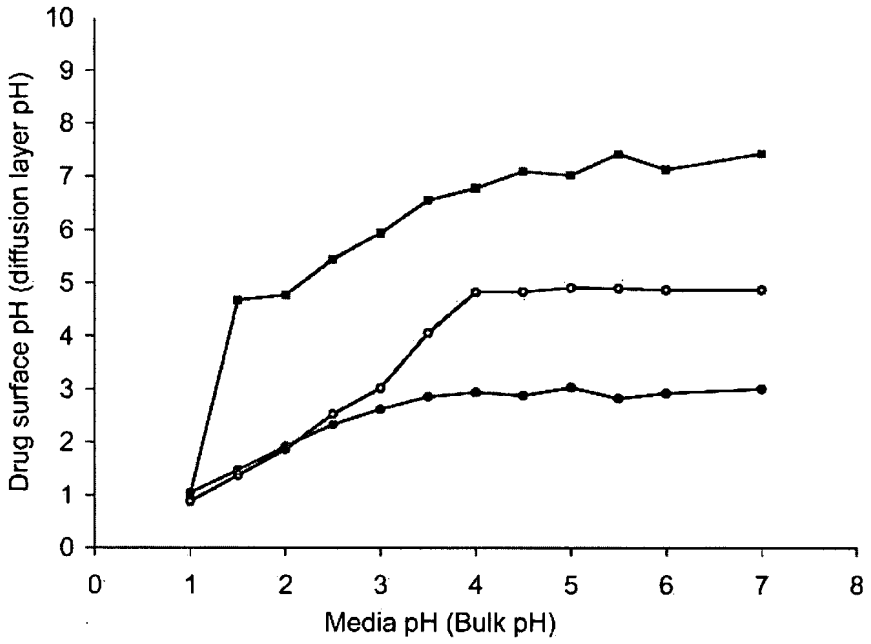

Fig. 9. pH at the surface of solid ($pH_{h=0}$) as a function of bulk pH of dissolution media (-●- haloperidol mesylate, -○- hydrochloride, and -■- free base).

salt form may well be preferred due to its ability to continuous higher dissolution at intestinal pH.

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